Phytoremediation: Effects of Timing on the Overall Health of Brassica Juncea

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1. Introduction

Phytoremediation is the process in which certain plants, known as accumulators, take heavy metals out of polluted soil and water environments, using their normal nutrient absorbing mechanisms. Phytoremediation is an emerging and environmentally safe technology aimed at tackling the problems of heavy-metal pollution that previous generations have left behind. This technology is fairly well researched, but there are still niche-areas that can be researched for different situations and situations that any scientist may run into in the real world.

The area of research that this experiment was designed to look at revolves around the aspect of timing of the application of the plants into the polluted soil. This research was designed to see if having the biomass (Brassica Juncea in these experiments) be exposed to the polluted soil at different stages of the Brassica Juncea's growth cycle. The hopes of this experiment were to use stand counts, overall health rating, and pictures taken over the course of this experiment to determine which stage of the growth cycle would be optimal for the plants growth and survival in the polluted soil. This optimization could lead to a higher-percentage of germinating and surviving plants that can absorb more pollutants out of the soil more efficiently.

The goal of this experiment is to test the hypothesis: The further that Brassica Juncea is along in its growth cycle when first exposed to lead, the healthier the Brassica Juncea will be. This will allow the Brassica Juncea to absorb more lead than its earlier exposed counterparts. One thing to note about this research experiment is that this procedure and hypothesis are different than the original, proposed experiment that was submitted in February of 2013. This will be addressed in the complication and issues section of this report.

2. Chronological Review of Time Spent on Project

Week	Tasks, Duties, Etc
1	Lab Orientation. Participated in ULearn Safety Classes.
2	Learned Pipetting Techniques. Prepared Knowns for AAS.
3	Learned how to use the AAS. More Preparing Knowns for AAS.
4	Purchasing and Acquiring of Supplies. Planting Test Brassica Juncea.
5	Recognition of Error in AAS. Attempted Recalibration of AAS. Buffer Testing
6	Disassembly of AAS. Attempted to Fix Leak. Discussed Solutions with Manufacturer.
7	Failure of AAS. Designed a New Experiment to Fit New Time Constraint.
8	Poisoned the Test Plants. Determined Kill Amount. Test Planted New Experiment.

9	Prepared Lead Solutions for New Experiment. Data Analysis and Finalized Design.
10	Planted Finalized Experiment. Prepared Future Lead Solutions for Experiment.
11	Data Collection. Photos. Overall Health Ranking.
12	Data Collection. Photos. Overall Health Ranking.
13	Data Collection. Photos. Overall Health Ranking.
14	Finished up Experiment. Data Analysis.
15	Clean up and Disposal of Plants and Soil. Disposed of Lead Solutions.
16	Prepared Lab Report and Presentation.

Figure 1: Chronological List of Time Spent on Project

3. Research Methodology and Theory

Design One: The Original Design

The Original Design of my experiment unfortunately had to be terminated 7 weeks into the semester. This was due to the lab's Atomic Absorption Spectrometer (AAS) breaking down and being at the stage where my mentor and I could not fix it within the time frame presented. This is discussed more in the Complication and Issues section. The original design was to have Brassica Juncea grown and then placed above different beakers of lead contaminated water with varying pH's. The experiment was designed to see if having a different pH of the solution, would affect the rate of accumulation. Solution samples were going to be taken, diluted, and ran through the AAS. This would allow us to back-calculate out the concentration of lead in the solution, to see which pH the plants were accumulating faster in. Due to the malfunction of the AAS, this experiment was terminated, and a new experiment was designed in its place to fit the short time frame that remained.

Design Two: The New Design

This new experiment required myself to look at three different design aspects of the experiment: Kill Concentration Design, Plant/Pot and Poisoning Design, and Growth Stage Timing.

Kill Concentration Design: In order to determine what I would use for the initial kill concentration, I pipetted concentrated lead solutions directly onto to root system of Brassica Juncea plants that were about 5 weeks into their growth cycle. I used 4 different lead solutions at 500, 1000, 1500, and 2000ppm Lead solutions made by using a premade lead reference solution. The only trial that was left standing was the 500ppm solution so that was going to be the initial kill concentration. This concentration was then tested to make sure that the plants could germinate in it. The new design was to have different concentrations of lead at 1/3, 2/3 and 3/3 of the kill concentrations, so some seeds were planted in these concentrations to make sure they would germinate. The only seeds that germinated were the 166 ppm group, so the experiment was redesigned to have that be the new kill concentration.

Figure 2: Experimental Lay-out

Name	Code	DAP*	Conc Lead in ppm
Control	0	N/A	0
1/3 Round 1	1	15	64
2/3 Round 1	2	15	128
3/3 Round 1	3	15	192
1/3 Round 2	4	25	64
2/3 Round 2	5	25	128
3/3 Round 2	6	25	192

Note: *DAP = Days After Planting

The actual planting set up itself was that of 16 seedlings planted in standard miracle grow potting soil. They were grown in 250mL plastic beakers, with 2cm of pea gravel on the bottom. The gravel was there so the water had some place to drain to and that the water didn't form a thick layer of mud in the bottom of the container. Each pot was watered every two days with 30mL of DI water, with the exception of the poisoning day. On that day, the plant was watered twice in one day, each time with 30mL of DI water and the respective amount of my premixed lead solution made from lead nitrate. This was done in order to be able to get the plants a little time to get some of the water out of the soil. Also this was done so the pot was not overloaded with water.

The days of 15 and 25 were chosen for a couple reasons. First, the true leaves on the Brassica Juncea started to dominate the cotyledons at 15 days. 25 days after planting were chosen for a couple reasons in itself. A lot of mass is added to the plants in that 10-day span. Notes taken during the first weeks of the test plants from weeks 4-9 noticed that the stems grew in length and added mass very rapidly during this week. Also, from the earlier testing done in the lab, the plants seemed to do most of their absorption of the lead within the first 4-5 days of introduction to the lead. This gave the last round of plants a good week to go through the majority of their absorption.

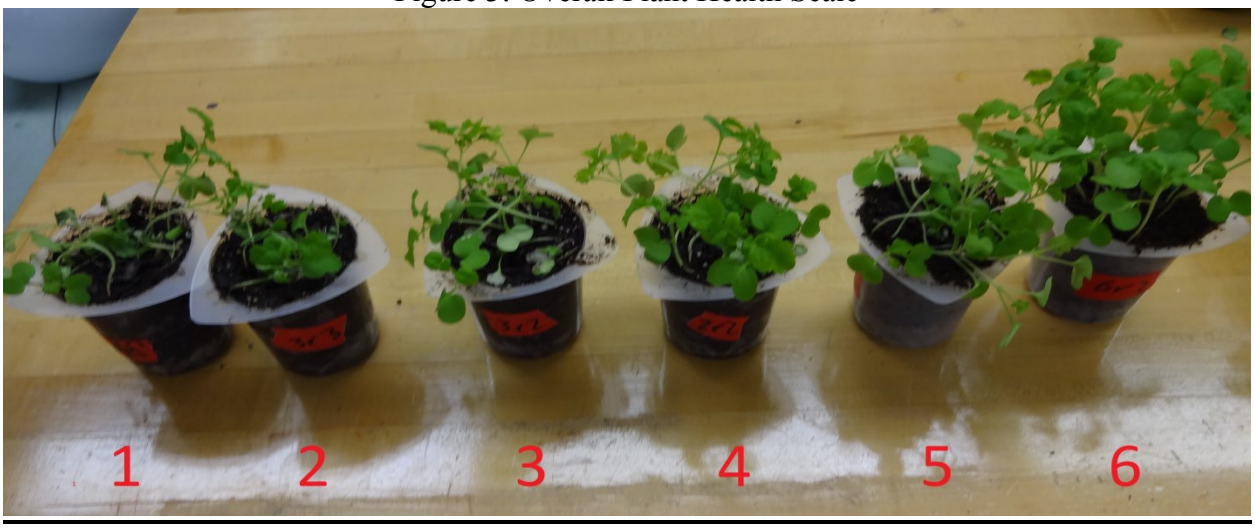
The original design of this experiment was to have seven different trials ran in triplicate in order to see how the plant's place in the growth-stage cycle affects how the plant reacts to different concentrations of lead. These concentrations of lead were determined due to trial and error that had occurred in the previous weeks to this experiments start. Above, Figure 2 describes the seven different trials that were run in this experiment.

4. Findings and Discussion

There were two main results that were gathered over the course of the four weeks of the experiment. The first result was that of an overall health rank on an overall health scale, based off of concepts that I have used at the University of Minnesota Northwest Research and Outreach Center. This scale is shown in in Figure 3. A score of six represents perfect health; all of the plants are alive and healthy. A score of one represents a very low stand count and the plants that

are alive being frail and smaller than normal plants. A 3 or 4 is the middle, where plants have started to die and/or the ones that are alive are a mix of healthy ones and ones with declining health. The second result was a stand count of the plants. The Brassica Juncea was counted as being alive if it exhibited certain characteristics (e.g. No vascular constriction on the stem, leaves green and health, and some turgor and rigidity in the plants themselves.).

Figure 3: Overall Plant Health Scale



Fibure 4: Stand Count of Brasica Juncea

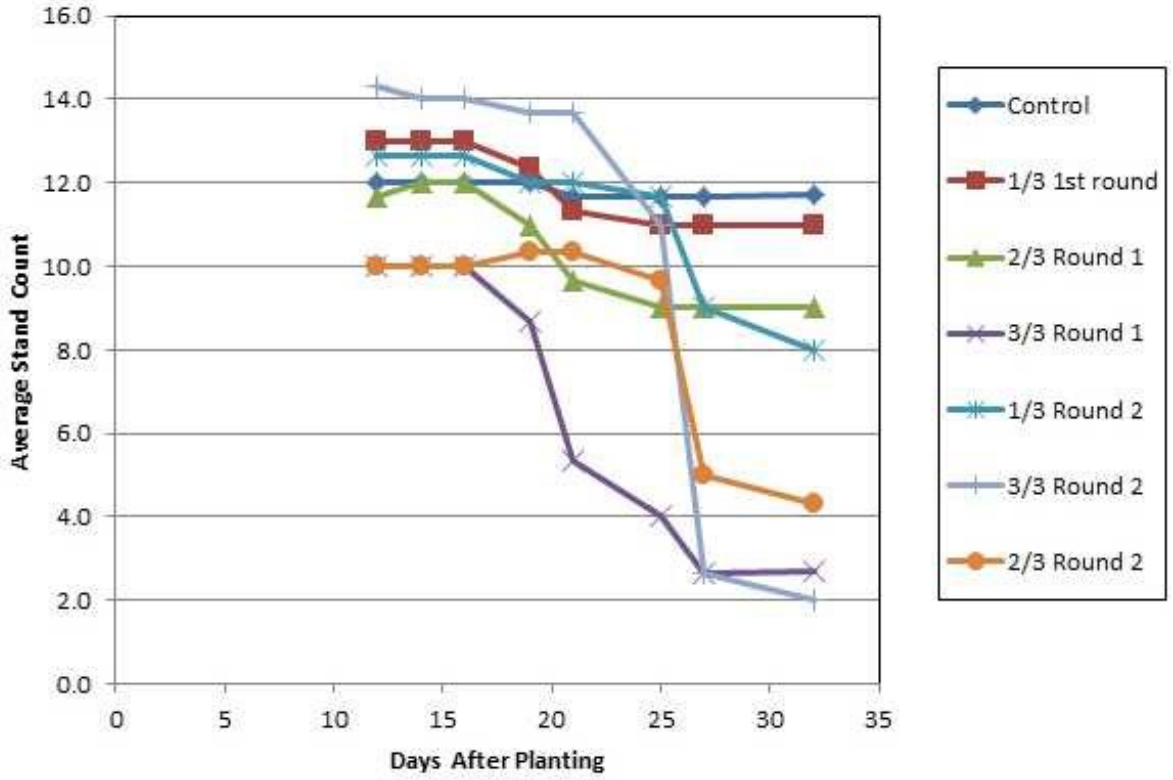


Figure 4 above shows the stand count of the tests. This data was taken every two days, unless situations arose in the data was unable to be taken.

Figure 5: Overall Health Score

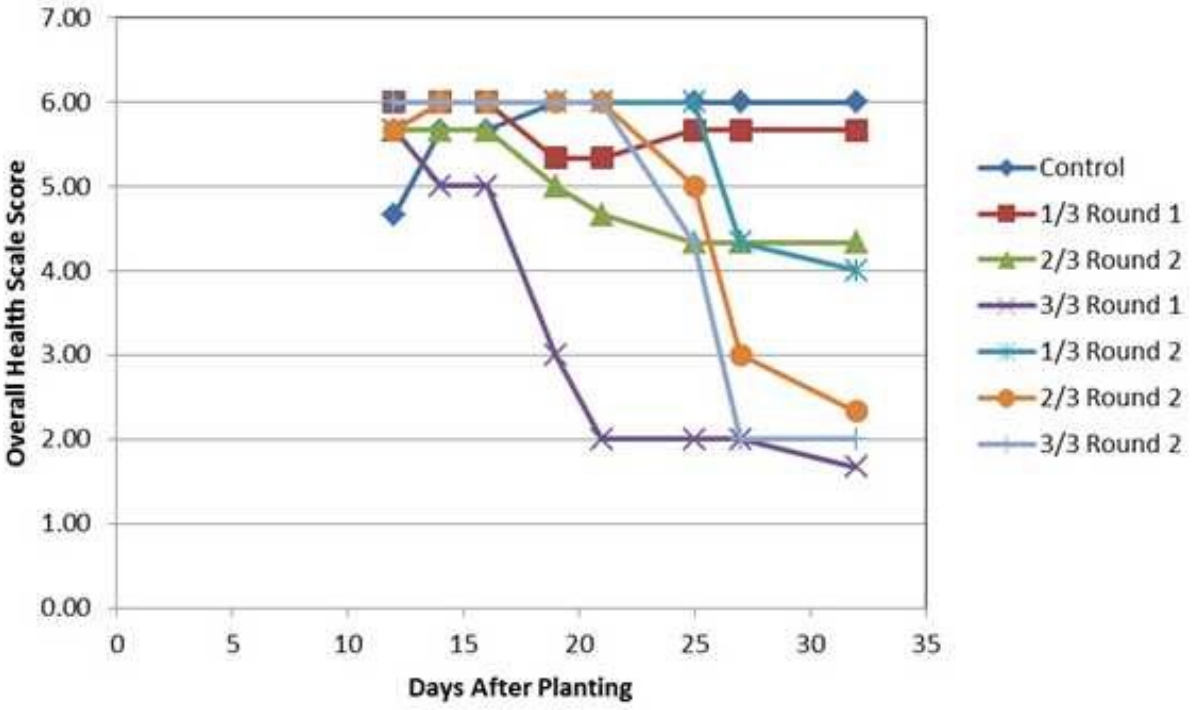


Figure 5 also shows the overall health scale scores of the trials. In both graphs, the values represent the mean value between the three replicates of each trial. There was no significant variance between the three replicates in any of the trials. Both of the graphs really show some interesting things about the data in both sets.

Figure 6: Comparing Graphs

	Ranked	Stand	Health Score
Best	1	Control	Control
	2	1/3 1st Round	1/3 1st Round
	3	2/3 1st Round	2/3 1st Round
	4	1/3 2nd Round	1/3 2nd Round
	5	2/3 2nd Round	2/3 2nd Round
	6	3/3 1st Round	3/3 2nd Round
Worst	7	3/3 2nd Round	3/3 1st Round

As you can see in Figure 6, along with the two graphs in Figure 4 and Figure 5, there is a relatively strong correlation between the two graphs. This means that it is likely that the two variables that were collected seem to have some effect on each other, which was expected.

One thing to pay attention to in this data is the order in which the trials ranked after the data was compared. The 1/3 concentration of both the 1st and 2nd round of planting were pretty close

together, but there was a major gap between the 2/3 concentration groups. Another thing to be noted is that the rate of decline in the 2nd round of poisoning seems to be much greater than that of the 1st round. This was against the original hypothesis that the older and more mature plants would not suffer from all of the ailments that the younger group did when they were poisoned. This was hypothesized because I thought that having a more developed vascular system and being older would allow the plants to be more resistant to the lead's negative effects. My new belief is that the older plants in this situation were growing so rapidly, that when they were trying to absorb the nutrients they need to grow, they also were taking up more lead than they were ready to accumulate. This makes me wish that the experiment would have had another 2 months to test this data. Then a set of plants could have been poisoned at 30 days, 45 days, and 60 days to see if this trend of growing too much would have also resulted in such drastic drops in stand and overall health.

From the data and observations that were gathered over the course of this experiment, I have concluded that the placement of plants at 25 days after planting into lead infested soils at relatively high lead concentrations (about 100ppm) would not be recommended over planting them at the true leave stage (15 days after planting). I wish that I would have been able to do more research on the topic. In that future research I would have liked to see how much longer you would have to let the brassica juncea grow before it will not experience such a drastic change in its overall health, therefore being able to be more efficient in its overall accumulation of lead.

5. Complications and Issues

5 weeks into the experiment, this experiment ran into a major issue. The Atomic Absorption Spectrometer (AAS) was not reading the known concentrations accurately at all. My mentor and I spent many hours attempting to fix the AAS. We decided to move onto a new experiment after repeated attempts of disassembling the AAS to clean it, attempted calibrations, changing of the bulbs used to detect the lead, and some e-mails with the manufactures. By the time we could fix the machine, I would not have had time to complete the UROP before the semester ended. With that, I had to scrap about 60 hours of previous work and design a new experiment that could be run in roughly six weeks that also didn't have the potential to fail. Then, I ran into another issue when I tried to determine the kill concentration of lead in the soil. The concentration that the plants grown during the attempted fixing of the AAS seemed to barely survive and function is was the maximum concentration I planted my new experiment's original seeds in for. This concentration, along with the 2/3 concentration were too high for the seedlings to germinate and grow. Even 1/3 of this concentration was still enough to barely allow the seeds to germinate. So I decided to redesign one last time where that new (1/3)*Kill Concentration would be the max concentration after the plants hit the true leaf stage. I ended up using 192ppm Lead solution made from lead nitrate to make this concentrated solution. This worked out nicely, but had delayed the project by another week.

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